wherein R_{11} is a straight or branched alkyl of from 1 to 6 carbon atoms, phenyl, or cycloalkyl having from 3 to 6 carbon atoms; R_{12} is hydrogen or methyl; and R_{13} is hydrogen, methyl, or carboxyl; individual enantiomers thereof; and pharmaceutically acceptable salts thereof.

REMARKS

Claims 38-51 remain in the application. Claims 38, 44, 45, 46, 47, 48, 49, 50, and 51 are in independent form. Claims 1-37 have been canceled.

The application is in condition for allowance, which allowance is respectfully solicited.

Respectfully submitted,

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2. The method of claim [46] 1 wherein R_{11} is a straight or branched alkyl group having from 1 to 6 carbon atoms.

3. The method of claim [46] 1 wherein the alkyl group has 4 carbon atoms.

4. The method of claim [46] 1 wherein R_{11} is isobutyl.

5. The method of claim [46] 1 wherein R_{13} is hydrogen.

6. The method of claim [46] 1 wherein R_{12} is hydrogen.

7. A method of treating a patient having psychotic disorders which includes administering to a patient an effective amount of the compound R-(-)-4-amino-3-(2-methylpropyl) butanoic acid.

8. A method of treating a patient having psychotic disorders which includes administering to a patient an effective amount of the compound S-(+)-4-amino-3-(2-methylpropyl) butanoic acid.

GABA AND L-GLUTAMIC ACID ANALOGS FOR ANTISEIZURE TREATMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of co-pending application U.S. Serial Number 886,080, filed May 20, 1992 which is a continuation-in-part of co-pending application U.S. Serial Number 618,692, filed November 27, 1990.

TECHNICAL FIELD

The present invention relates to novel compounds that are analogs of glutamic acid and gamma-aminobutyric acid (GABA). More specifically, the analogs are useful as antiseizure therapy for central nervous system disorders such as epilepsy, Huntington's chorea, cerebral ischemia, Parkinson's disease, tardive dyskinesia, and spasticity. It is also possible that the present invention could be used as an antidepressant, anxiolytic, and antipsychotic activity.

BACKGROUND OF THE INVENTION

Gamma aminobutyric acid (GABA) and glutamic acid are two major neurotransmitters involved in the regulation of brain neuronal activity. GABA is the major inhibitory neurotransmitter and L-glutamic acid is an excitatory transmitter (Roberts E, et al, GABA in Nervous System Function, Raven Press: New York, 1976; McGeer EG, et al, Glutamine, Glutamate, and GABA in the Central Nervous System; Hertz L, Kvamme E, McGeer EG,

Schousbal A, eds., Liss: New York, 1983;3-17). An imbalance in the concentration of these neurotransmitters can lead to convulsive states. Accordingly, it is clinically relevant to be able to control convulsive states by controlling the metabolism of this neurotransmitter. When the concentration of GABA diminishes below a threshold level in the brain, convulsions result (Karlsson A, et al, Biochem. Pharmacol 1974;23:3053-3061). When the GABA levels rise in the brain during convulsions, seizures terminate (Hayashi TJ, Physiol. (London) 1959;145:570-578). The term seizure as used herein means excessive unsynchronized neuronal activity that disrupts normal neuronal function. In several seizure disorders there is concomitant with reduced brain GABA levels a diminished level of L-glutamic acid decarboxylase (GAD) activity also observed (McGeer PO, et al, In: GABA in Nervous System Function; Roberts E, Chase TN, Tower DB, eds., Raven Press: New York 1976:487-495; Butterworth J, et al, Neurochem. 1983;41:440-447; Spokes EG, Adv. Exp. Med. Biol. 1978;123:461-473; Wu JY, et al, Neurochem. Res. 1979;4:575-586; and Iversen LL, et al, Psychiat. Res. 1974;11:255-256). Often, the concentrations of GAD and GABA vary in parallel because decreased GAD concentration results in lower GABA production.

Because of the importance of GABA as an inhibitory neurotransmitter, and its effect on convulsive states and other motor dysfunctions, a variety of approaches have been taken to increase the brain GABA concentration. For example, the most obvious approach was to administer GABA. When GABA is injected into the brain of a convulsing animal, the convulsions cease (Purpura DP, et al, Neurochem. 1959;3:238-268). However, if GABA is administered systematically, there is no anticonvulsant effect because GABA, under normal

circumstances, cannot cross the blood brain barrier (Meldrum BS, et al, Epilepsy; Harris P, Mawdsley C, eds., Churchill Livingston: Edinburg 1974:55. In view of this limitation, there are three alternative approaches that can be taken to raise GABA levels.

The most frequent approach is to design a compound that crosses the blood brain barrier and then inactivates GABA aminotransferase. The effect is to block the degradation of GABA and thereby increase its concentration. Numerous mechanism-based inactivators of GABA aminotransferase are known (Silverman RB, Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology, Vol. I and II, CRC: Boca Raton 1988).

Another approach is to increase GABA concentrations in the brain by making GABA lipophilic by conversion to hydrophobic GABA amides (Kaplan JP, et al, G.J. Med. Chem. 1980;23:702-704; Carvajal G, et al, Biochem. Pharmacol. 1964;13:1059-1069; Imines: Kaplan JP, Ibid.; or GABA esters: Shashoua VE, et al, J. Med. Chem. 1984;27:659-664; and PCT Patent Application WO85/00520, published 2/14/85) so that GABA can cross the blood brain barrier. Once inside the brain, these compounds require amidase and esterases to hydrolyze off the carrier group and release GABA.

Yet another approach is to increase brain GABA levels by designing an activator of GAD. A few compounds have been described as activators of GAD. The anticonvulsant agent, maleicimid, was reported to increase the activity of GAD by 11% and as a result increase GABA concentration in the substantia nigra by up to 38% (Janssens de Varebeke P, et al, Biochem. Pharmacol. 1983;32:2751-2755. The anticonvulsant drug sodium valproate (Loscher W, Biochem. Pharmacol. 1982;31:837-842; Phillips NI, et al, Biochem. Pharmacol. 1982;31:2257-2261) was also reported to activate GAD and increase GABA levels.

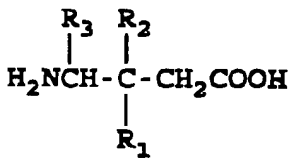
The compounds of the present invention have been found to activate GAD in vitro and have a dose dependent protective effect on-seizure in vivo.

Also, the compounds of the present invention have been found to bind a novel binding site which was identified to bind tritiated gabapentin. Gabapentin has been found to be an effective treatment for the prevention of partial seizures in patients refractory to other anticonvulsant agents. Chadwick D, Gabapentin, pp. 211-222, In: Recent Advances in Epilepsy, Vol. 5, Pedley TA, Meldrum BS, (eds.) Churchill Livingstone, New York (1991). The novel binding site labeled by tritiated gabapentin was described in membrane fractions from rat brain tissue and in autoradiographic studies in rat brain sections, Hill D, Ibid. This binding site has been used to evaluate the compounds of the present invention.

The novel compounds of the present invention are set forth below as Formula I. It should be noted that the compound of Formula I wherein R_1 is methyl and each of R_2 and R_3 is hydrogen is taught in Japan Patent Number 49-40460.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided compounds of the Formula I



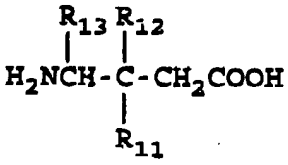
I

wherein R_1 is a straight or branched alkyl of from 1 to 6 carbons, phenyl or cycloalkyl having from 3 to 6 carbon atoms; R_2 is hydrogen or methyl; and R_3 is

hydrogen, methyl, or carboxyl; with the proviso that when each of the R_2 and R_3 is hydrogen, R_1 is other than methyl. Pharmaceutically acceptable salts of the compounds of Formula I are also included within the scope of the present invention. Also included within the scope of the present invention are the individual enantiomeric isomers of the compounds of the Formula I.

The present invention also provides pharmaceutical compositions of the compounds of Formula I.

Also provided as a part of the present invention is a novel method of treating seizure disorders in a patient by administering to said patient an anticonvulsant effective amount of a compound of the following Formula II



II

wherein R_{11} is a straight or branched alkyl of from 1 to 6 carbon atoms, phenyl, or cycloalkyl having from 3 to 6 carbon atoms; R_{12} is hydrogen or methyl; and R_{13} is hydrogen, methyl, or carboxyl; or an individual enantiomeric isomer thereof; or a pharmaceutically acceptable salt thereof.

Also, the present invention provides a method for increasing brain neuronal GABA and provides pharmaceutical compositions of the compounds of Formula II.

The present invention provides novel processes for the synthesis of chiral Formula I compounds.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a series of 3-alkyl-4-aminobutyric acid or 3-alkyl glutamic acid analogs which are useful as anticonvulsants. Illustrative of the alkyl moieties as represented by R_1 and R_{11} in Formulas I and II are methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, isopentyl, and neopentyl as well as other alkyl groups. The cycloalkyl groups represented by R_1 and R_{11} in Formulas I and II are exemplified by cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. The analogs are further shown herein to prevent seizure while not causing the side effect of ataxia, such a side effect being found in several anti-seizure pharmaceuticals.

The more preferred compounds of the present invention are of Formula I above wherein R_3 is hydrogen, R_2 is hydrogen, and R_1 isobutyl.

That is, the preferred compound is 4-amino-3-(2-methylpropyl)butanoic acid. It has been found that this compound is unexpectedly more potent than the other analogs synthesized in accordance herewith and tested in vivo. What is further surprising, as the following data shows, is that this preferred compound is the least effective one of the analogs tested in activating GAD in vitro. Accordingly, it was very unexpected that this preferred compound had such a high potency when tested in vivo.

The most preferred compounds of the present invention are the (S)-(+)- and the (R)(-)-4-amino-3-(2-methylpropyl)butanoic acid with the (S)-(+)-enantiomer being most preferred. The (S)-(+)-enantiomer was found to be the most potent compound within the scope of the present invention for displacement of tritiated gabapentin, and both the

(S)-(+)- and the (R)-(-)-enantiomers showed pronounced stereoselectivity for both displacement of tritiated gabapentin and for anticonvulsant activity in vivo.

The compounds made in accordance with the present invention may form pharmaceutically acceptable salts with both organic and inorganic acids or bases. For example, the acid addition salts of the basic compounds are prepared either by dissolving the free base in aqueous or aqueous alcohol solution or other suitable solvents containing the appropriate acid and isolating the salt by evaporating the solution. Examples of pharmaceutically acceptable salts are hydrochlorides, hydrobromide, hydrosulfates, etc, as well as sodium, potassium, and magnesium, etc, salts.

The method for the formation of the 3-alkyl-4-aminobutanoic acids starting from 2-alkanoic esters is prepared from commercially available aldehydes and monomethyl malonate by the Knoevenagel reaction, (Kim YC, et al, J. Med. Chem. 1965:8509) with the exception of ethyl 4,4-dimethyl-2-pentenoate.

More specifically, the following is a procedure which can be generally applied to the preparation of all the 3-alkylglutamic acids. Ten grams of a 3-alkyl-5,5-dicarbethoxy-2-pyrrolidinone was refluxed in 150 mL of 49% fuming HBr for 4 hours. After this time, the contents were placed in an evaporator and the volatile constituents were removed in vacuo with the aid of a hot-water bath. The gummy residue was dissolved in 25 mL of distilled water and the water was removed with the aid of the evaporator. This process was repeated once more. The residue was dissolved in 20 mL of water, and the pH of the solution was adjusted to 3.2 with concentrated NH_3 solution. At this point the chain length of the individual 3-alkylglutamic acids altered the solubility so that those whose side chains were larger precipitated with the ease from solution.

Precipitation of the alkylglutamic acids with smaller substituents (methyl, ethyl, and propyl) could be encouraged by cooling on an ice bath or by diluting the aqueous solution with 100 mL of absolute ethanol. Precipitation from the water-alcohol mixture is complete in 48 hours. Care must be taken to add the ethanol slowly to prevent the precipitation of an amorphous solid which is not characteristic of the desired 3-alkylglutamic acids. Samples of the amino acids were purified for analysis by recrystallizing from a water-ethanol mixture. All melted with decomposition. Melting points of the decomposed 3-alkylglutamic acids corresponded with those of their pyroglutamic acids.

Ethyl 4,4-dimethyl-2-pentenoate was prepared from 2,2-dimethylpropanol and ethyl lithioacetate, followed by dehydration of the β-hydroxy ester with phosphoryl chloride and pyridine.

The Michael addition of nitromethane to alpha, β-unsaturated compounds mediated by 1,1,3,3-tetramethylguanidine or 1,8-diazabicyclo-[5.4.0]undec-7-ene(DBU) afforded 4-nitroesters in good yields. More specifically, a mixture of nitromethane (5 mol), α,β-unsaturated ester (1 mol), and tetramethyl-guanidine (0.2 mol) was stirred at room temperature for 2 to 4 days. (In case of methyl acrylate, the ester has to be added at a temperature below 300.) The progress of the reaction was followed by IR (disappearance of the C=C band) and G.L.C. analysis. The reaction mixture was washed with dilute hydrochloric acid and extracted with ether. The organic extract was dried, the solvent removed at reduced pressure, and the 20 residue distilled at a pressure of 2 torr. Although the aliphatic nitro compounds are usually reduced by either high pressure catalytic hydrogenation by metal-catalyzed transfer

hydrogenation, or by newly introduced hydrogenolysis methods with ammonium formate or sodium borohydride and palladium as catalysts, applicants have found that 4-nitrocarboxylic esters can be reduced almost quantitatively to the corresponding 4-aminocarboxylic esters by hydrogenation using 10% palladium on carbon as catalysts in acetic acid at room temperature and atmospheric pressure. The amino esters produced were subjected to acid hydrolysis to afford the subject inventive compounds in good yields. This procedure provides access to a variety of 3-alkyl-4-aminobutanoic acids as listed in Tables 1 and 2 as examples and thus is advantageous in comparison to methods previously used.

Examples of more specific methods of making compounds in accordance with the present invention are as follows, optionally utilizing the methods described in detail above. When the starting material is not commercially available, the synthetic sequence may be initiated with the corresponding alcohol, which is oxidized to the aldehyde by the method of Corey EJ, et al, Tetrahedron Lett. 1975:2647-2650.

The chiral compounds of Formulas I and II are prepared as set forth in the schematic in Chart I hereof. Although the schematic in Chart I depicts the chiral synthesis of specific compound (S)-(+)-4-amino-3-(2-methylpropyl)butanoic acid, one skilled in the art can readily see that the method of synthesis can be applied to any diastereomeric compound of Formulas I and II.

In Chart I Ph is phenyl, Bn is benzyl, THF is tetrahydrofuran, LDA is lithium diisopropylamide, $BH_3 \cdot SMe_2$ is borane dimethyl sulfide complex, TsCl is tosyl chloride, and DMSO is dimethylsulfoxide.

The detailed synthetic procedure is set forth hereinbelow in Example 1. The key introductory

literature for this methodology was discussed in Evans' paper, J. Am. Chem. Soc. 1982;104:1737-9. The metal enolate can be formed with a lithium or sodium amide base, and subsequently alkylated to give an a substituted carboxylic acid derivative. This methodology was valuable for the enantioselective synthesis of these α-substituted carboxylic acid derivatives. In this seminal paper, Evans described the preparation of propionic acid derivatives with a series of simple alkylating agents. By varying the stereochemistry of the chiral synthon (the oxazolidinone), he was able to get high stereoselectivity.

Evans has used this chiral auxiliary in other synthetic studies, but none has been related to 4-amino-3-(2-methylpropyl)butanoic acid which contains a β-substituted-γ-amino acid. The methodology as presented by Evans teaches toward α-substitution, and away from β-substitution, and has not been used in the preparation of this type of unusual amino acid. N-acyloxazolidinones have been used to form chlorotitanium enolates that have been reacted with Michael adducts such as acrylonitrile, J. Org. Chem. 1991;56:5750-2. They have been used in the synthesis of the rutamycin family of antibiotics, J. Org. Chem. 1990;55:6260-8 and in stereoselective aldol condensations, Org. Synth. 1990;68:83-91. Chiral α-amino acids were prepared via the oxazolidinone approach. In this sequence, a dibutylboron enolate was brominated and displaced with azide, Tetrahedron Lett. 1987;28:1123-6. Other syntheses of β-hydroxy-α-amino acids were also reported via this chiral auxiliary through aldol condensation (Tetrahedron Lett. 1987;28:39-42; J. Am. Chem. Soc. 1987;109:7151-7). α,β-Unsaturated N-acyloxazolidinones have also been used to induce chirality in the Diels-Alder reaction,

(J. Am. Chem. Soc. 1988;110:1238-56. In none of these examples, or others found in the literature, is this methodology used to prepare (β-substituted carboxylic acids or 3-substituted GABA analogs.

In another embodiment, the chiral compounds of Formulas I and II can be prepared in a manner which is similar to the synthesis depicted in Chart I. In this embodiment, however, step 8 in Chart I is replaced by an alternate two step procedure which is set forth hereinbelow in Example 2 (sodium hydroxide is preferred, however, other solvents known to those of skill in the art which can hydrolyze the azide (8) to intermediate azide (8a) can be employed). Instead of reducing the azide (8) to the amino acid (9) in Chart I, the alternate procedure hydrolyzes the azide (8) to give an intermediate azide (8a) which is subsequently reduced (see Chart Ia).

There are two major advantages to hydrolyzing azide (8) to give the intermediate azide (8a) prior to reduction. The first advantage is that intermediate azide (8a) may be purified by extraction into aqueous base. After the aqueous extract is acidified, intermediate azide (8a) may be extracted into the organic phase and isolated. This allows for a purification of intermediate azide (8a) which does not involve chromatography. The purification of azide (8) requires chromatography which is very expensive and often impractical on a large scale.

The second advantage is that intermediate azide (8a) may be reduced to amino acid (9) without added acid. Reduction of azide (8) requires addition of acid, e.g., hydrochloric acid in order to obtain amino acid (9). Unfortunately, lactamization of amino acid (9) is promoted by the presence of acid. Intermediate azide (8a) may be reduced under near neutral conditions

to give amino acid (9), thus minimizing the problem of lactam formation.

In another preferred embodiment, the chiral compounds of Formulas I and II can be prepared as set forth in the Schematic in Chart II hereof. Although the schematic in Chart II depicts the chiral synthesis of specific compound (S)-(+)-4-amino-3-(2-methyl-propyl)butanoic acid, one skilled in the art can readily see that the method of synthesis can be applied to any diastereomeric compound of Formulas I and II.

In Chart II Ph is phenyl, and Ts is tosyl.

The detailed synthetic procedure is set forth hereinbelow in Example 3. This procedure is similar to the synthesis route depicted in Chart I, however, the procedure of Chart II replaces the benzyl ester in the synthesis route of Chart I with a t-butyl ester. The desired amino acid (9) and (109) is the same end product in both Charts I and II, respectively.

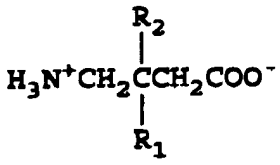
There are several advantages to using the t-butyl ester rather than the benzyl ester in the synthesis of amino acid (9) or (109). A first advantage relates to the hydrolysis of the chiral auxiliary in step 4 of Chart 1. During the hydrolysis of the chiral auxiliary in this reaction some hydrolysis of the benzyl ester often occurs. Hydrolysis of the t-butyl ester in Chart II has not been experienced.

Another advantage relates to the use of alcohol (106) in Chart II over the use of alcohol (6) in Chart I. A problem with the benzyl ester-alcohol is the tendency of the benzyl ester-alcohol to undergo lactonization as shown below. Although lactonization of the benzyl ester can be avoided under some conditions, the t-butyl ester-alcohol is far less prone to lactonization.

The above is a significant finding because the compound having the least ability to activate GAD in vitro surprisingly had an approximately 10-fold increase in potency over the other compounds tested. Even more unexpected is the absence of ataxic side effect coupled to this increase in potency.

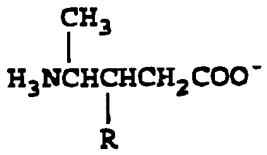
TABLE 1

Activation of GAD by GABA analogs at various concentrations expressed in %



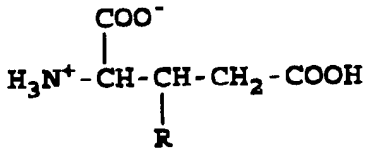
R_1, R_2	2.5 mM	1.0 mM	0.5 mM	0.25 mM	0.1 mM	0.05 mM
(R,S)-CH_3,H	239	168	142	128	118	107
(R)-CH_3H	327	202	185	135	128	109
(S)-CH_3H	170	118	--	103	--	--
CH_3, CH_3	174	125	--	109	--	--
(R,S)-C_2H_5,H	172	128	--	108	--	--
(R,S)-n-C_3H_7,H	156	112	--	105	--	--
(R,S)-i-C_3H_7,H	140	108	--	104	--	--
(R,S)-n-C_4H_9,H	178	117	--	105	--	--
(R,S)-i-C_4H_9,H	143	113	--	109	--	--
(R,S)-s-C_4H_9,H	169	119	--	105	--	--
(R,S)-t-C_4H_9,H	295	174	147	121	117	108
(R,S)-neo-C_5H_{11},H	279	181	--	130	--	--
(R,S)-i-C_5H_{11},H	142	118	--	109	--	--
(R,S)-C_6H_{11},H	125	100	--	100	--	--
(R,S)-C_6H_5,H	218	129	--	110	--	--

TABLE 1 (Cont.)



R	2.5 mM	1.0 mM	0.5 mM	0.25 mM	0.1 mM	0.05 mM
H(R,S)	140	111	--	104	--	--
H(R)	173	125	--	108	--	--
H(S)	100	100	--	100	--	--
CH_3	143	121	--	109	--	--
C_6H_5	207	151	--	112	--	--
Sodium Valproate	207	138	124	119	115	105
GABAPENTIN	178	145	--	105	--	--

Activation of GAD by glutamate analogs expressed in %



R	2.5 mM	1.0 mM	0.25 mM
CH_3	212	144	113
C_2H_5	170	128	113
n-C_3H_7	153	125	108
i-C_3H_7	144	114	105
n-C_4H_9	133	117	105
i-C_4H_9	129	112	106
C_6H_5	172	135	112
Sodium Valproate	207	138	119

TABLE 2

Prevention of tonic extensor seizures in mice following intravenous administration of 3-substituted GABA derivatives

R	Dose (mg/kg)	Time After Dose (min)	Effect # Protected/ # Tested	Ataxia # Ataxia/ # Tested
(R,S)-CH_3	10	120	0/5	0/5
	30	120	4/5	0/5
	100	120	3/5	0/5
CH_3	1	120	1/10	0/10
	3	120	2/10	0/10
	10	120	4/10	0/10
	30	120	3/10	0/10
	100	120	3/10(5/10)	1/10
CH_3	10	120	1/10	1/10
	30	120	2/10	0/10
	100	120	5/10	0/10
t-C_4H_9	10	120	2/10	0/10
	30	120	2/10	0/10
	100	120	5/10	0/10
C_2H_5	3	120	1/5	0/5
	10	120	1/5	0/5
	30	120	2/5	0/5
	100	120	5/5	0/5
$(CH_3)_2$	30	120	4/5	0/5
	100	120	4/5	0/5
n-C_4H_9	10	120	1/10	0/10
	30	120	3/10	0/10
	100	120	4/10	0/10

TABLE 2 (Cont.)

R	Dose (mg/kg)	Time After Dose (min)	Effect # Protected/ # Tested	Ataxia # Ataxia/ # Tested
s-C_4H_9	3	120	2/10	0/10
	10	120	3/10	0/10
	30	120	2/10	0/10
i-C_4H_9	0.3	120	1/10	0/10
	0.8	120	3/10	0/10
	2.0	120	5/10	0/10
	5.5	120	7/10	0/10
	14.4	120	9/10	0/10
n-C_3H_7	3	120	2/10	0/10
	10	120	2/10	3/10
	100	120	3/10	0/10
i-C_3H_7	10	120	5/10	1/10
	30	120	5/10	0/10
	100	120	6/10	0/10
C_6H_5	100	120	0/10	0/10
neo-C_5H_{11}	10	120	2/10	0/10
	30	120	4/10	0/10
	100	120	4/10	0/10

- High-intensity corneal electroshock consisted of 50 mA, base-to-peak sinusoidal current for 0.2 seconds. All other data was from low-intensity electroshock, 17 mA base-to-peak sinusoidal current for 0.2 seconds.

TABLE 3

Threshold maximal electroshock with isobutyl GABA

Time of Testing	# Protected
1 hr.	2/10
2 hr.	8/10
4 hr.	4/10
8 hr.	2/10

TABLE 4

Threshold maximal electroshock with isobutyl GABA

Dose m/k	# Protected
0.3	1/10
0.8	3/10
2.0	5/10
5.5	7/10
14.4	9/10

TABLE 5

Maximal electroshock data

R	Dose (mg/kg)	Time After Dose (min)	Effect # Protected/ # Tested	Ataxia # Ataxia/ # Tested
i-C_4H_9	10	120	1/5	0/5
i-C_4H_9	30	120	4/5	0/5
i-C_4H_9	100	120	4/5	0/5

As noted hereinabove, the S-(+)enantiomer of 4-amino-3-(2-methylpropyl)butanoic acid (3-isobutyl GABA or IBG) which is structurally related to the known anticonvulsant, gabapentin, potently displaces tritiated gabapentin from a novel high-affinity site in rat brain membrane fractions. Also the S-(+)enantiomer of 3-isobutyl GABA is responsible for virtually all blockade of maximal electroshock seizures in mice and rats. The R(-) enantiomer of 3-isobutyl GABA is much less effective in the blockade of maximal electroshock seizures and in displacement of tritiated gabapentin from the novel high-affinity binding site. Table 6 below sets forth data comparing gabapentin, racemic 3-isobutyl GABA ((±)-IBG), S-(+)-3-isobutyl GABA ((S)-IBG) and R-(-)-3-isobutyl GABA ((R)-IBG) in these assays.

TABLE 6

Test System	3-Isobutyl GABA (ED_{50})			
	Gabapentin	(±)-IBG	(S)-IBG	(R)-IBG
Gabapentin Receptor Binding (IC_{50})	0.14 μM	0.10 μM	0.044 μM	0.86 μM
IV Mouse Low-Intensity Electroshock	4.3 mg/Kg	4.8 mg/Kg	4.0 mg/Kg	>100 mg/Kg
IV Mouse Maximal Electroshock	75 mg/Kg	10 mg/Kg	18 mg/Kg	>100 mg/Kg
PO Mouse Maximal Elctroshock	200 mg/Kg	47 mg/Kg	12 mg/Kg	
IV Mouse Ataxia	>100 mg/Kg (IP)	>100 mg/Kg	>300 mg/Kg	>100 mg/Kg

Time course of anticonvulsant activity (all compounds) peaks 2.0 hours after dose and mostly gone 8 hours after dose.

The data set forth in Table 6 was obtained as follows. For anticonvulsant testing, male CF-1 strain mice (20-25 g) and male Sprague-Dawley rats (75-115 g) were obtained from Charles River Laboratories and were maintained with free access to food and water before testing. Maximal electroshock was delivered with corneal electrodes by conventional methods (Krall, supra, 1975) except that low-intensity electroshock with mice consisted of 17 mA of current rather than the conventional 50 mA (zero to peak). Briefly, mice were given test substance and were tested for prevention of seizures by application of electrical current to the corneas by 2 metal electrodes covered with gauze and saturated with 0.9% sodium chloride. Electroshock stimulation was delivered by a constant-current device that produced 60 Hz sinusoidal electrical current for 0.2 seconds. For rats, maximal electroshock stimulation consisted of 120 mA of current. Ataxia in mice was assessed by the inverted screen procedure in which mice were individually placed on a 4.0-inch square of wire mesh that was subsequently inverted (Coughenour, supra, 1978). Any mouse that fell from the wire mesh during a 60 second test period was rated as ataxic. ED_{50} values were determined by probit analysis of results with at least 5 dose groups of 10 mice or 8 rats each.

All drugs were freely soluble in aqueous media. For in vivo studies, drug solutions were made in 0.9% sodium chloride and given in a volume of 1 mL/100 g body weight. Intravenous administration was given by bolus injection into the retro-orbital sinus in mice. Oral administrations were by intragastric gavage.

For binding studies, partially purified synaptic plasma membranes were prepared from rat neocortex using sucrose density gradients. The cerebral cortex of 10 rats was dissected from the rest of the brain and

homogenized in 10 volumes (weight/volume) of ice-cold 0.32 M sucrose in 5 mM tris-acetate (pH 7.4) using a glass homogenizer fitted with a teflon pestle (10-15 strokes at 200 rpm). The homogenate was centrifuged at 100 g for 10 minutes and the supernatant collected and kept on ice. The pellet (PI) was rehomogenized in 20 mL of tris-sucrose and the homogenate recentrifuged. The combined supernatants were centrifuged at 21,500 g for 20 minutes. The pellet (P2) was resuspended in 1.2 M tris-sucrose and 15 mL of this mixture was added to ultracentrifuge tubes. On to this, 10 mL of 0.9 M sucrose was layered followed by a final layer of 5 mM tris-acetate, pH 8.0. Tubes were centrifuged at 100,000 g for 90 minutes. The synaptic plasma membranes located at the 0.9/1.2 M sucrose interface were collected, resuspended in 50 mL of 5 mM tris-acetate, pH 7.4, and centrifuged at 48,000 g. The final pellet was resuspended in 50 mL of tris-acetate, pH 7.4, aliquoted, and then frozen until use.

The assay tissue (0.1 to 0.3 mg protein) was incubated with 20 mM [^{3}H]-gabapentin in 10 mM HEPES buffer (pH 7.4 at 20°C, sodium free) in the presence of varying concentrations of test compound for 30 minutes at room temperature, before filtering onto GFB filters under vacuum. Filters were washed 3 times with 5 mL of ice cold 100 mM NaCl solution and dpm bound to filters was determined using liquid scintillation counting. Nonspecific binding was defined by that observed in the presence of 100 mM gabapentin.

In view of the above demonstrated activity of the compounds characterizing the present invention and in particular the 4-amino-3-(2-methylpropyl)butanoic acid (isobutyl GABA) the compounds made in accordance with the present invention are of value as pharmacological

agents, particularly for the treatment of seizures in mammals, including humans.

EXAMPLE 1

(S)-(+)-4-amino-3-(2-methylpropyl)butanoic acid

The following "steps", refer to Chart I.

Step 1

To a solution of 4-methylvaleric acid (50.0 g, 0.43 mol) in 100 mL of anhydrous chloroform was added thionyl chloride (60 mL, 0.82 mol). The reaction mixture was refluxed for 2 hours and then cooled to room temperature. Excess chloroform and thionyl chloride was removed by distillation. The residue oil was then fractionally distilled to give 45.3 g (78%) of the acid chloride (2), bp = 143-144°C.

Acid chloride (2) was also be prepared by an alternative method which eliminated use of chloroform which has waste disposal and operator exposure difficulties. The alternate method also minimized the formation of 4-methylvaleric anhydride.

To a solution of thionyl chloride (98.5 kg, 828 mol) and N,N-dimethylformamide (2 kg, 27 mol) was added 4-methylvaleric acid (74 kg, 637 mol) while maintaining a reaction temperature of 25-30°C. Hexanes (30 L) were added and the solution was maintained at 30-35°C for 1 hour and 15 minutes. The solution was then heated to 70-75°C for 1 hour and 10 minutes. The solution was subjected to atmospheric distillation until a solution temperature of 95°C was reached. After cooling, hexanes (30 L) were added and the solution was subjected to atmospheric distillation until a solution temperature of 97°C was reached. Distillation of the residual oil produced 79 kg (92%) of acid chloride (2), bp = -77°C, 60-65 mm Hg.

Step 2

To a solution of (4R,5S)-(+)-4-methyl-5-phenyl-2-oxazolidinone (5.27 g, 29.74 mmol) in 70 mL of anhydrous tetrahydrofuran at -78°C under argon atmosphere was added a 1.6 M solution of n-butyllithium (19 mL, 30.40 mmol) in hexanes slowly. The mixture was allowed to stir at -78°C for 15 minutes then the acid chloride (4.5 g, 33.43 mmol) was added to quench the reaction. The reaction was stirred at -78°C for 10 minutes then 0°C for 30 minutes. A saturated solution of sodium bicarbonate (50 mL) was added and the mixture was stirred at 0°C for 30 minutes. The organic layer was collected and the aqueous layer was extracted with ethyl acetate (3x). The organic extracts were combined and dried with anhydrous magnesium sulfate. It was then filtered and concentrated to give a colorless oil. The oil was then chromatographed with 8% ethyl acetate in hexanes on silica gel to give 7.56 g (82%) of the acyloxazolidinone (3) as a white solid.

Anal. Calcd for $C_{16}H_{21}NO_3$:
C, 69.79; H, 7.69; N, 5.09.
Found: C, 69.56; H, 7.63; N, 5.06.

Acyloxazolidinone (3) was also prepared by an alternate method which was conducted at -5°C to 0°C rather than -78°C which is difficult and expensive to achieve on a manufacturing scale. The alternate method also gave a crystalline solid from the reaction mixture rather than an oil which must be chromatographed.

To a solution of 4-methyl-5-phenyl-2-oxazolidinone (64 g, 0.36 mol) in anhydrous tetrahydrofuran (270 g) at -5°C was added a 15% solution of n-butyllithium in hexane (160 g, 0.37 mol) over a temperature range of -5°C to 0°C. Acid chloride (2) (48.6 g, 0.36 mol) was added at -10°C to 0°C. The reaction was quenched with a solution of water (90 mL) and sodium bicarbonate

(4 g). Ethyl acetate (200 g) was added and the layers were separated. The organic layer was extracted with water (2 x 50 mL) and the aqueous phases were back extracted with ethyl acetate (100 g). The organic extracts were combined and approximately 150 mL of solvent was removed by distillation. Atmospheric distillation was continued and heptane (2 x 200 g) was added until a vapor temperature of 95°C was reached. The solution was cooled to 5°C. The product was collected by filtration, washed with cold heptane, and dried to give 79 g (80%) of acyloxazolidinone (3).

Step 3

To a solution of diisopropylamine (4.8 mL, 34.25 mmol) in 30 mL of anhydrous tetrahydrofuran at 0°C under argon atmosphere was added a 1.6 M solution of n-butyllithium (21 mL, 33.60 mmol) in hexanes slowly. The solution was stirred at 0°C for 30 minutes then cooled to -78°C. A solution of the acyloxazolidinone (3) (7.56 g, 27.46 mmol) in 30 mL of anhydrous tetrahydrofuran was added and the pale yellow solution was stirred at -78°C for 30 minutes. Benzyl α-bromoacetate was added and the resulting solution was stirred at -25°C for 2 hours. The reaction mixture was quenched with a half-saturated ammonium chloride solution and extracted by ethyl acetate (2x). The combined organic layers were dried with anhydrous magnesium sulfate and then filtered and concentrated to give a colorless oil. The oil was then chromatographed with 8% ethyl acetate in hexanes on silica gel to give 6.16 g (53%) of the acyloxazolidinone (4) as a white solid.

Anal. Calcd for $C_{25}H_{29}NO_5$:
C, 70.90; H, 6.90; N, 3.31.
Found: C, 70.47; H, 6.87; N, 3.45.